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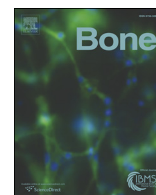
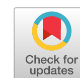


Full Length Article

Perimenopausal bone histomorphometry before and after menopause



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ABSTRACT

Investigators and clinicians have had few normal bone histomorphometry data available to compare with those found in diseased patients, or in the results of treatments. The Goals and Objectives of this work are two-fold: 1. to present static and dynamic bone histomorphometry data from transilial bone biopsies performed on 76 healthy, premenopausal women. 2. To present paired static and dynamic bone histomorphometry data from bone biopsies on a subset ($N = 51$ pairs) of these same healthy women whose biopsies were repeated 12 months after their last menses. Statistical comparisons between the pre- and postmenopausal data are presented. These data will shrink this important gap, both for clinicians and investigators.

We enrolled 76 healthy, premenopausal women over age 46, performed transilial bone biopsies after tetracycline labeling, and during a period of 9.5 years, we re-biopsied 51 of them who passed through menopause and remained healthy the entire time. We also obtained serum biochemical measurements, and serial DXA exams during the period of observation. The dynamic bone histomorphometry demonstrated a doubling of bone remodeling, and increases in serum bone markers at the time of the second biopsy. Lumbar spine bone density also declined, and there were significant correlations between serum markers and histomorphometry variables. The data demonstrate that healthy menopause results in an important increase in bone remodeling, and a loss of bone density. We do not fully understand the mechanisms of these transmenopausal changes, but the data provide some clues that are helpful.

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1. Introduction

Menopause is the event in women's lives most closely linked to bone loss and subsequent risk of osteoporosis and attendant fractures later in life. Albright first recognized the role of estrogen deficiency in the bone loss of menopause [1] and characterized it as a failure of bone formation. Frost characterized the nature of bone remodeling in adult humans as a "quantum-based" phenomenon [2] in which packets of bone are resorbed, and then replaced in sequence. Heaney et al. [3], using radiocalcium kinetics, demonstrated that the global effect of menopause on the skeleton was to increase the rate of remodeling markedly. Thus, increase in remodeling rates at menopause have been demonstrated by methods (radio-calcium kinetics) other than histomorphometry, and thus is well documented. The combined results of these early studies suggested that the mechanism for bone loss at menopause is an increase in the rate of appearance of bone remodeling sites along with a failure of the bone formation process to replace completely the bone removed during resorption at these sites.

Bone histomorphometry of iliac crest biopsy specimens provides a means to examine directly, in living humans, the bone cell and tissue changes associated with skeletal integrity at menopause and later in life. Since the 1970s, the study of bone remodeling by histomorphometry of iliac crest biopsies has matured, and a benchmark of this maturation, along with the origination of many of the ideas of the investigators involved, is available [3].

One of the handicaps in using histomorphometry of transiliac biopsies in the study of all bone diseases in humans, including postmenopausal osteoporosis, is the shortage of normal reference data from cross sectional and longitudinal human studies. This is particularly true for normal premenopausal, and early postmenopausal, women. Several good studies are available [4–15], but the sample sizes are generally small, the results show considerable variation, and there are few serial longitudinal studies available. Variation among the studies can be due to differences in selections of the human subjects, in the formulae calculating the variables, in combining wide age ranges of study subjects, and other factors. Nevertheless, there seems to be agreement that bone remodeling increases at menopause, and trabecular bone volume (and osteonal wall thickness) decreases with age. To our knowledge, the study reported here is the only one that includes a substantial number of biopsies in the same healthy subjects both before, and 12 months after onset of menopause. Some data from these subjects have been

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reported [7,16,17]. In this report, we describe the entire set of static and dynamic bone histomorphometry data from a group of 76 healthy premenopausal women, 51 of whom had repeat transiliac biopsies 12 months after their last menses in the absence of hormone replacement, or other factors that might confound the results. The data contribute normal reference information on healthy premenopausal white women over age 46. They confirm that bone remodeling increases almost immediately after the onset of menopause, and that bone structural loss begins as well.

2. Materials and methods

2.1. Human Subjects

Recruitment began in March of 1988 and was complete by December 1989. Recruitment was accomplished by word of mouth and community advertising. We screened 224 women to enroll a convenience sample of 78 healthy white women into a study to determine bone tissue- and cell-level changes in bone remodeling that occur at menopause in healthy women [17]. The reasons for declining entry were mostly personal i.e. fear of radiation exposure (DXA), inability to attend all the visits, and other personal reasons. Past history of pregnancy was not recorded, and current use of contraceptives was an exclusion factor. The ultimate goal was to provide a normal reference database for iliac bone histomorphometry in healthy perimenopausal women. The study design called for transiliac bone biopsy after tetracycline labeling on entry into study and, again, 12 months after the last menses. Entry criteria included ≥46 years of age and menses during each of the prior 2 years. Entry also required that serum levels of estradiol (E_2) be >50 pg/ml, and follicle stimulating hormone (FSH) be <25 mIU/ml on samples obtained between the 17th and 25th day of the menstrual cycle. There were a large number of diagnoses in the exclusion criteria [17]. We excluded anyone pregnant, or with a diagnosis or treatment that would affect bone health. We documented good health by history, physical and clinical laboratory examination. The Creighton Institutional Review Board approved the study and all subjects provided written consent prior to entry.

Study visits occurred at 6-month intervals throughout the 9.5 years of observation. There were no pregnancies during the 9.5 years of observation. We defined the date of menopause as the date of the last menses that was followed by 12 months without menses. The definition also required a serum $E_2 \leq 20$ pg/ml and FSH >75 mIU/ml at 12 months of absent menses. Bone densitometry by dual photon absorptiometry (DXA), serum and urine biochemical measurements (bone biomarkers), and health histories were obtained at each visit.

Of the 78 women who enrolled, 77 biopsies were performed, one biopsy could not be performed because of adipose problems, and one biopsy was completed but could not be used because bone particles were present in the marrow space caused by the teeth in the trephine, leaving 76 premenopausal entry biopsies. Fifty one of these women remained healthy and underwent natural menopause during observation without hormone replacement therapy, or other confounding events. Twenty five subjects were not included in the group whose data were used in the pre-post menopause comparisons. The reasons for exclusion were that they were placed on hormone replacement therapy before menopause by their private physicians, usually to treat menopausal symptoms, they did not pass through menopause during the 9.5 years of observation, or underwent total hysterectomy and used hormone replacement intermittently so the date of menopause was not definable. The 25 subjects not included in the postmenopausal data analyses did not drop out. All but one could not be included in the postmenopausal biopsies because they did not become estrogen deplete i.e., they did not have menopause, or they had hormone replacement therapy prescribed by their personal physician. The only dropout was one subject who moved out of the area. Nevertheless, all but that one dropout continued in the study, and underwent all the measurements except the biopsy [17].

2.2. Measurements

Hormones: Initially, for the perimenopausal women, we obtained serum specimens for measurement of E_2 and gonadotropin levels between the 17th and 25th day of the menstrual cycle. Later, when irregular bleeding intervals supervened, we obtained serum samples at times unrelated to menstrual cycles. Details of the assay methods used in this study have been previously published [17].

Serum and urine biochemistry methods: We measured urine creatinine (Cr) with a Gilford Impact 400 (Gilford Systems, Oberlin, OH, USA), an automated system based on a modified Jaffe reaction with a lower limit of detection of 0.06 mg/dl. Serum bone-specific alkaline phosphatase was measured in Baylink's lab using wheat germ agglutinin precipitation, heat inactivation, and a two-site immuno-radiometric assay [18]. We used a semi-automated adaptation of the manual colorimetric method of Bergman and Loxely [19] to measure hydroxyproline (Hypro) in 2-h urine samples that we collected after a 12-h fast. The lower limit of detection for this assay was 1.7 μmol/l. Serum 25(OH)D was measured by the Diasorin method.

Transiliac biopsy: each subject received in vivo double tetracycline labeling and a transiliac biopsy, 7.5 mm in diameter, was performed as previously described [6,7,20]. The oral tetracycline schedule was 250 mgs four times daily, three days on, 14 days off, three more days on and then biopsy 5–14 days later (3-14-3; 5-14) as previously described [20]. The specimen was embedded in methylmethacrylate, and sectioning began about 250 μm into the specimen. Then two groups of nine sections, each group >250 μm apart, were read from the central part of each biopsy core. Five sections in each group were 5 μm, and 4 were 8 μm in thickness. In 51 who underwent two biopsies, the time gap between biopsies averaged 5.34 years, with standard deviation of 2.05 years. The median was 5.00 years, the 2.5th %tile was 1.69 years, and the 97.5th %tile was 9.05 years. These time gaps were sufficient to distinguish between the two labeling periods in the biopsy specimens.

Measured and calculated variables in trabecular bone histomorphometry used here are described elsewhere [21]. Each width measurement was corrected by an obliquity factor ($\pi/4$) in order to be converted to an expression of thickness and be used in various histomorphometry calculations. The entry biopsy was performed on the right side of the pelvis in each case, and the second biopsy was performed on the opposite side. Each biopsy was performed at a point about 2 cm posterior and inferior to the anterior-superior spine. The second biopsy occurred >2 years after the first in all but 2 subjects (1.58 years and 1.59 years).

Here we give a compact technical explanation of the histomorphometric variables. The Trabecular Number, TbN (#/mm), is the average number of trabeculae encountered as one moves linearly across the slides/sections in any direction. The more trabeculae, the more robust is the structure. The Trabecular Thickness, TbTh (μm), is derived from the ratio of bone volume per total volume (BV/TV) to bone surface density (BS/TV), allowing for units in each case. Intuitively, the thicker the trabeculae, the stronger the whole bone. Trabecular Spacing, TbSp (μm), is derived by apportioning the distance along survey lines of the trabeculae and then subtracting the trabecular thickness. The Bone Volume to Tissue Volume ratio, BV/TV (%), is derived from the ratio of the total trabecular bone area to the total tissue area, including the marrow space. Wall Thickness, WTh (μm), is the average distance between cement lines and quiescent, completed trabecular surfaces, multiplied by a factor of $\pi/4$ to correct for obliquity, the various "slants" at which one views this distance [6]. WTh actually measures the average width of completed remodeling sites. Osteoid Thickness, OTh (μm), is the average perpendicular distance between the trabecular surface of unmineralized osteoid, and the mineralized bone, corrected for obliquity ($\pi/4$). Osteoid volume per bone volume, OV/BV (%), derived from the

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